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THE EFFECTS OF SELECTED HYDRAZINES UPON FISH AND INVERTEBRATES

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The toxicity of hydrazine, monomethylhydrazine, and 1-1, dimethylhydrazine was ascertained from static LC50 estimations using two freshwater fish, golden shiners and channel catfish, and two invertebrates, amphipods and isopods. The data did not clearly indicate that the invertebrates were more sensitive than the fish to the chemicals and that the relative toxicities of the hydrazines were different. However, 1-1, dimethylhydrazine was generally the least toxic to both the invertebrates and fish. | | |

PREFACE

This study was conducted in the Toxic Hazards Division, Environmental Quality Branch, Air Force Aerospace Medical Research Laboratory. The research was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations," Task 04, Workunit 18, from June 1979 until September 1979. This project was supported, in part, by Laboratory Directors Fund (78-9).

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INTRODUCTION

Hydrazine (Hz), monomethylhydrazine (MMH), and 1-1 dimethylhydrazine (UDMH) are currently used as Air Force rocket propellants. Hz is also utilized as an emergency power supply for the Air Force's F-16 and in commercial enterprises as antioxidants and organic derivatives (Back et al., 1978). Hz, MMH, and UDMH are very water soluble and capable of achieving pronounced effects upon freshwater organisms (Henderson and Pickering, 1959; Heck et al., 1962; Heck et al., 1963; Hoover et al., 1964; Greenhouse, 1976; Slonim, 1977; and Fisher et al., 1978). Consequently, environmental effects of Hz, MMH, and UDMH have become important areas of investigation.

The purpose of this study was to determine static LC50s (median lethal concentration) of three hydrazines, Hz, MMH, and UDMH, to freshwater invertebrates and fish.

MATERIALS AND METHODS

Static 48h bioassays and one static 72h bioassay were conducted with the invertebrates and static 96h bioassays were conducted with the fish (A.P.H.A., 1976).

Test organisms: Two freshwater invertebrates, isopods (Asillidae) and amphipods (*Hyaella azteca*), were used as the test organisms. Both the isopods and the amphipods were obtained from the Carolina Biological Co., Burlington, North Carolina. The invertebrates were acclimated at least four days in water comparable to the bioassay water. The invertebrates were fed plant material.

Two species of fish, *Ictalurus punctatus* Rafinesque, the channel catfish, and *Notemigonus crysoleucas* (Mitchell), the golden shiner, were used. All fish were obtained from Fender's Fish Hatchery, Baltic, Ohio. The fish were acclimated for at least five days in water similar to the bioassay water. The fish were fed TetraMin® until two days before testing. The mean weights and lengths (n=30) of the channel catfish were 4.76g \pm 1.17 and 7.97 cm \pm 0.93, respectively. The golden shiner's mean weight and length (n=15) were 1.41g \pm 0.60 and 5.8 cm \pm 6.9, respectively. Both the invertebrates and the fish were maintained in a sequential 14h photoperiod and 10h of darkness.

Test water: The tap water was purified using a reverse osmosis (R.O.) membrane filtering system after pretreatment with two-1.0 micron rope filters, a charcoal filter, and a water softener. The clean product water from the R.O. was diluted with charcoal-treated tap water to restore the electrolyte content to a desired level. Table 1 lists the preexposure water quality for the bioassays. Hydrogen ion (pH) was measured daily (Table 4) and dissolved oxygen and CaCO₃ hardness were measured intermittently throughout the studies.

TABLE 1
PREEXPOSURE WATER QUALITY

| <u>Bioassay</u> | <u>Trial</u> | <u>Temperature °C</u> | <u>Hardness (ppm)</u> | <u>Dissolved Oxygen (ppm)</u> |
|-----------------|--------------|-----------------------|-----------------------|-------------------------------|
| Amphipods | 1 | 22.5 | 132 | 7.0 |
| | 2 | 21.0 | 96 | 7.2 |
| Isopods | 1 | 24.0 | 96 | 7.4 |
| | 2 | 23.0 | 96 | 7.3 |
| Catfish | 1 | 22.0 | 106.4 | 7.2 |
| | 2 | 22.5 | 112.8 | 7.0 |
| Shiner | 1 | 21.0 | 140 | 7.4 |
| | 2 | 22.5 | 172.8 | 6.6 |

Equipment: 2ℓ Pyrex beakers were used as exposure chambers for the invertebrate studies and fish bioassays were conducted in 15 gallon aquaria, six of which were plexiglass, and nine glass. Acclimation tanks for the fish and invertebrates consisted of a "Living Stream" and a 15 gallon aquarium, respectively.

Instruments used included a Gilford 240 spectrophotometer, a Corning Model 12 pH meter, a Yellow Springs Model 54 oxygen meter, and a Mettler E200 top-loading balance.

Toxicants: The test chemicals include Hz (Eastman, +95%), MMH (Aldrich, +98%), and UDMH. The UDMH was redistilled at Edwards Air Force Base. It contained 0.0001% N-nitrosodimethylamine and 0.18% dimethylamine. Formaldehyde dimethylhydrazine and water were not detected (<.01%).

Fresh toxicant stock solutions (Table 5) were prepared, volume to volume, with bioassay water for each study by delivering neat toxicant into volumetric flasks with either a gas tight syringe or, for the higher concentrations in the fish bioassays, with an Oxford Sampler[®] pipette. In one case, golden shiner, Trial 2, UDMH was delivered with a 10ℓ volumetric pipette. Appropriate concentrations were then delivered, volume to volume to the exposure aquaria, which contained 1.5ℓ and 30ℓ of bioassay water for the invertebrate and fish bioassays, respectively.

Toxicant concentrations during bioassays were determined daily with colorimetric procedures. Dimethylaminobenzaldehyde (DMBA) was used as the color reagent for Hz and MMH. The absorbance values for Hz were read after 20 minutes at 458 nm¹; MMH was read after 30 minutes of color development at 485 nm (Reynolds and Thomas, 1964), UDMH concentrations were determined using 0.1% trisodium pentacyanaminoferroate solution according to the method described by Pinkerton (1961). Absorbance was read at 500 nm after 60 minutes.

¹Stephen Klein, University of California, Berkeley, personal communication.

Pretrial calibration curves for Hz and MMH in the invertebrate bioassays ranged from 0.1 to 1.0 ppm and the UDMH calibration curve ranged from 1.0 to 50 ppm. In the fish studies, Hz and MMH calibration curves ranged from 0.15 to 3.0 ppm and the UDMH calibration curve ranged from 1.5 to 6.0 ppm. At least five concentrations of each chemical were used to obtain each calibration curve. Because of the high linearity of the pretrial calibration curves, subsequent calibration curves for the bioassays were completed with only three concentrations, within the pretrial ranges for each curve. If the toxicant concentrations were above the calibration range, the toxicant was diluted to within the calibration range.

LC50 estimations (Litchfield and Wilcoxon, 1949 and moving average-angle¹) were compared by direct observation of the range of 95% confidence intervals (A.P.H.A., 1976) to ascertain relative toxicity.

RESULTS AND DISCUSSION

Invertebrate bioassay: Hz was more toxic than MMH and UDMH to the amphipods and no differences in toxicity occurred with MMH and UDMH (Table 2). The isopods were less sensitive to UDMH than Hz and MMH and no differences in toxicity were evident with Hz and MMH (Table 2). The amphipods were more sensitive to Hz than the isopods and MMH and UDMH appeared to be equally toxic to both invertebrates (Table 2).

Fish bioassays: The catfish were the most sensitive to Hz, followed by MMH, then UDMH. The golden shiners were equally affected by Hz, MMH, and UDMH was the least toxic (Table 3). The catfish and golden shiner responses to Hz and MMH were similar and UDMH was more toxic to the catfish than to the golden shiner.

These toxicity data did not clearly suggest that invertebrates were more sensitive than fish to the hydrazines or that the relative toxicity of these chemicals were different. The fish and isopods lethal responses to Hz were similar, while the amphipods were very sensitive to Hz. It does appear that UDMH was generally less toxic to the invertebrates and fish than MMH and Hz. This was true for the isopods and fish. Hz was more toxic than MMH to the amphipods and catfish, while Hz and MMH were equally toxic to the isopods and golden shiners.

There are several common shortcomings in interpretation of static bioassays for an accurate LC50 estimation (e.g. toxicant loss, decomposition, Table 6, and build-up of metabolic wastes). We experienced another problem, excessive deaths in some of the invertebrate controls (Table 7). The former problems could be reduced by using a continuous-flow design and the latter problem by better handling technique.

¹ The computer program for this method was obtained from the Environmental Protection Agency, Newtown Fish Toxicology Station, Cincinnati, Ohio.

TABLE 2

LC50 Values for Invertebrate Studies

Amphipods

| <u>Trial</u> | <u>Compound</u> | <u>48 hr LC50 ppm</u> | <u>Confidence Intervals (95%)</u> |
|--------------|-----------------|-----------------------|-----------------------------------|
| 2 | Hz | 0.04 | 0.01 - 0.12 |
| 2 | MMH | 1.20 | 0.40 - 3.60 |
| 1 | UDMH | 4.70 | 2.04 - 10.80 |

Isopods

| | | | |
|---|------|-------------------|--------------|
| 1 | Hz | 1.30 ^a | 0.42 - 4.02 |
| 2 | MMH | 0.82 | 0.30 - 2.30 |
| 1 | UDMH | 12.40 | 7.20 - 21.10 |

Comparisons of LC50 Values

Between ChemicalsAmphipodsCompounds ComparedSignificant Difference*

| | <u>yes</u> | <u>no</u> |
|----------|------------|-----------|
| Hz/MMH | x | |
| Hz/UDMH | x | |
| MMH/UDMH | | x |

Isopods

| | | |
|----------|---|---|
| Hz/MMH | | x |
| Hz/UDMH | x | |
| MMH/UDMH | x | |

Between Organisms

| | | |
|-----------|---|---|
| Hz/Hz | x | |
| MMH/MMH | | x |
| UDMH/UDMH | | x |

a 72h LC50

* Significant differences are determined by nonoverlapping of 95% confidence intervals.

TABLE 3

LC50 Values for Fish Studies

Catfish

| <u>Trial</u> | <u>Compound</u> | <u>96h LC50</u> | <u>Confidence Intervals (95%)</u> |
|--------------|-----------------|-----------------|-----------------------------------|
| 1,2 | Hz | 1.00 | 0.32 - 2.07 |
| 1,2 | MMH | 3.54 | 2.51 - 4.97 |
| 1,2 | UDMH | 11.35 | 5.18 -18.73 |

Shiner

| | | | |
|-----|------|-------|--------------|
| 1,2 | Hz | 1.12 | 0.57 - 1.84 |
| 1,2 | MMH | 2.27 | 1.50 - 3.96 |
| 1,2 | UDMH | 34.00 | 27.30 -43.60 |

Comparisons of LC50 Values

Between Chemicals

| <u>Catfish</u> | <u>Compounds Compared</u> | <u>Significant Difference</u> * | |
|----------------|---------------------------|---------------------------------|-----------|
| | | <u>Yes</u> | <u>No</u> |
| | Hz/MMH | X | |
| | Hz/UDMH | X | |
| | MMH/UDMH | X | |
| <u>Shiner</u> | Hz/MMH | | X |
| | Hz/UDMH | X | |
| | MMH/UDMH | X | |

Between Organisms

| | | |
|-----------|---|---|
| Hz/Hz | | X |
| MMH/MMH | | X |
| UDMH/UDMH | X | |

* Significant differences are determined by nonoverlapping of 95% confidence intervals.

TABLE 4

pH of Test Water During Bioassays

Amphipods (Trial 1)

| Compound Nominal Concentration (ppm) | Time (hrs) | | |
|---|------------|-----|-----|
| | 0 | 24 | 48 |
| Hz | | | |
| 0.5 | 7.9 | 7.9 | 8.0 |
| 1.0 | 7.9 | 8.0 | 8.0 |
| 5.0 | 8.1 | -* | - |
| 10.0 | 8.3 | - | - |
| 50.0 | 8.7 | - | - |

MMH

| | | | |
|-------|-----|-----|-----|
| 1.0 | 7.8 | 7.7 | 7.2 |
| 5.0 | 7.9 | - | - |
| 10.0 | 8.1 | - | - |
| 50.0 | 8.5 | - | - |
| 100.0 | 8.7 | - | - |

UDMH

| | | | |
|-------|-----|-----|-----|
| 5.0 | 7.5 | 7.6 | 7.6 |
| 10.0 | 7.7 | 7.8 | 7.8 |
| 25.0 | 7.7 | 7.8 | 7.9 |
| 50.0 | 8.0 | 8.0 | 8.0 |
| 100.0 | 8.2 | 8.2 | - |

Control

| | | | |
|---|-----|-----|-----|
| 1 | 7.8 | 7.9 | 8.0 |
| 2 | 7.8 | 7.8 | 8.0 |

Amphipods (Trial 2)

| | | | |
|-----|-----|-----|-----|
| Hz | | | |
| .01 | 7.3 | 7.6 | 7.7 |
| .05 | 7.5 | 7.7 | 7.9 |
| .10 | 7.6 | 7.8 | 7.9 |
| .25 | 7.6 | 7.8 | 8.0 |
| .50 | 7.7 | 7.9 | 8.0 |

* No pH values recorded, 100% mortality.

TABLE 4 (Cont.)

Amphipods (Trial 2, cont.)

| Compound | Nominal Concentration (ppm) | Time (hrs) | | | |
|----------|-----------------------------|------------|-----------|-----------|-----------|
| | | <u>0</u> | <u>24</u> | <u>48</u> | <u>72</u> |
| MMH | | | | | |
| | .01 | 7.7 | 7.8 | 7.9 | |
| | .05 | 7.7 | 7.7 | 7.9 | |
| | .1 | 7.7 | 7.8 | 8.0 | |
| | .25 | 7.7 | 7.8 | 8.0 | |
| | .5 | 7.8 | 7.9 | 8.0 | |
| Control | | | | | |
| | 1 | 7.1 | 7.4 | 7.7 | |
| | 2 | 7.6 | 7.8 | 7.8 | |

Isopods (Trial 1)

| | | | | | |
|-----------|-----|-----|-----|-----|-----|
| Hydrazine | | | | | |
| | .01 | 6.5 | 6.9 | 7.2 | 7.6 |
| | .05 | 6.5 | 7.0 | 7.5 | 7.6 |
| | .1 | 6.5 | 7.0 | 7.5 | 7.7 |
| | .5 | 6.6 | 7.1 | 7.6 | 7.8 |
| | 1.0 | 6.7 | 7.2 | 7.6 | 7.8 |
| MMH | | | | | |
| | .01 | 6.7 | 7.1 | 7.6 | |
| | .05 | 6.7 | 7.1 | 7.3 | |
| | .1 | 6.7 | 7.1 | 7.4 | |
| | .5 | 6.7 | 7.1 | 7.5 | |
| | 1.0 | 6.8 | 7.2 | 7.4 | |
| UDMH | | | | | |
| | 5 | 7.1 | 7.3 | 7.6 | |
| | 10 | 7.1 | 7.5 | 7.7 | |
| | 25 | 7.2 | 7.6 | 7.8 | |
| | 50 | 7.5 | 7.7 | 7.9 | |
| | 100 | 7.7 | 7.8 | 7.9 | |
| Control | | | | | |
| | 1 | 6.9 | 7.3 | 7.6 | 7.7 |
| | 2 | 6.9 | 7.3 | 7.6 | 7.7 |

Isopods (Trial 2)

| | | | | |
|-----|-----|-----|-----|-----|
| MMH | | | | |
| | .05 | 6.5 | 7.1 | 7.4 |
| | .5 | 6.8 | 7.2 | 7.6 |
| | 1.0 | 6.9 | 7.3 | 7.7 |

TABLE 4 (Cont.)

Isopods (Trial 2, cont.)

| Compound | Nominal Concentration (ppm) | Time (hrs) | | | | |
|----------|-----------------------------|------------|-----------|-----------|-----------|-----------|
| | | <u>0</u> | <u>24</u> | <u>48</u> | <u>72</u> | <u>96</u> |
| MMH | | | | | | |
| 5.0 | | 7.0 | 7.4 | 7.8 | | |
| 10.0 | | 7.2 | 7.5 | 7.8 | | |
| 25.0 | | 7.7 | 7.7 | 7.9 | | |
| Control | | 7.0 | 7.1 | 7.7 | | |

Catfish (Trial 1)

| | | | | | | |
|-----------|--|-----|-----|-----|-----|-----|
| Hydrazine | | | | | | |
| .1 | | 7.5 | 7.2 | 6.9 | 7.2 | 7.0 |
| 1.0 | | 7.6 | 7.3 | 7.0 | 7.3 | 7.1 |
| 10.0 | | 8.1 | - | - | - | - |
| 25.0 | | 8.4 | - | - | - | - |
| 50.0 | | 8.6 | - | - | - | - |

| | | | | | | |
|------|--|-----|-----|-----|-----|-----|
| MMH | | | | | | |
| .1 | | 6.7 | 6.9 | 6.8 | 7.1 | 7.0 |
| 1.0 | | 6.7 | 7.0 | 6.9 | 7.2 | 7.1 |
| 10.0 | | 7.0 | 7.2 | - | - | - |
| 25.0 | | 7.7 | - | - | - | - |
| 50.0 | | 8.0 | - | - | - | - |

| | | | | | | |
|------|--|-----|-----|-----|-----|-----|
| UDMH | | | | | | |
| 5 | | 7.1 | 6.9 | 7.0 | 7.2 | 7.1 |
| 10 | | 7.4 | 7.2 | 7.1 | 7.2 | 7.1 |
| 25 | | 7.9 | 7.4 | 7.2 | - | - |
| 50 | | 8.0 | 7.5 | - | - | - |
| 100 | | 8.2 | 7.7 | - | - | - |

| | | | | | | |
|---------|--|-----|-----|-----|-----|-----|
| Control | | | | | | |
| 1 | | 7.2 | 7.0 | 7.1 | 7.0 | 6.9 |
| 2 | | 7.5 | 7.2 | 7.0 | 7.0 | 6.9 |

Catfish (Trial 2)

| | | | | | | |
|-----------|--|-----|-----|-----|-----|-----|
| Hydrazine | | | | | | |
| .1 | | 6.7 | 6.9 | 7.1 | 7.1 | 7.1 |
| .5 | | 6.6 | 6.9 | 7.1 | 7.1 | 7.1 |
| 1.0 | | 6.6 | 6.9 | 7.1 | 7.1 | 7.0 |
| 5.0 | | 6.9 | - | - | - | - |
| 10.0 | | 7.3 | - | - | - | - |

TABLE 4 (Cont.)

Catfish (Trial 2, cont.)

| Compound | Time (hrs) | | | | |
|------------------------------------|------------|-----------|-----------|-----------|-----------|
| <u>Nominal Concentration (ppm)</u> | <u>0</u> | <u>24</u> | <u>48</u> | <u>72</u> | <u>96</u> |
| MMH | | | | | |
| 1.0 | 6.9 | 7.0 | 7.1 | 7.1 | 7.1 |
| 2.5 | 6.8 | 7.0 | 7.1 | 7.2 | 7.1 |
| 5.0 | 6.8 | 7.0 | 7.0 | - | - |
| 7.5 | 6.9 | 7.0 | - | - | - |
| 10.0 | 7.0 | 7.1 | - | - | - |
| UDMH | | | | | |
| 5 | 7.0 | 7.1 | 7.1 | 7.1 | 7.1 |
| 10 | 7.1 | 7.2 | 7.2 | 7.2 | 7.2 |
| 15 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 |
| 20 | 7.2 | 7.3 | 7.2 | 7.2 | 7.3 |
| 25 | 7.3 | 7.4 | 7.3 | 7.3 | 7.4 |
| Control | | | | | |
| 1 | 6.5 | 7.0 | 6.9 | 6.9 | 7.0 |
| 2 | 6.6 | 7.1 | 7.0 | 7.0 | 7.0 |

Shiner (Trial 1)

| | | | | | | |
|-----------|-----|-----|-----|-----|-----|-----|
| Hydrazine | | | | | | |
| .1 | 6.8 | 6.9 | 7.2 | 7.3 | 7.3 | 7.3 |
| .5 | 6.8 | 6.9 | 7.2 | 7.3 | 7.5 | 7.5 |
| 1.0 | 6.8 | 6.8 | 7.2 | 7.3 | 7.6 | 7.6 |
| 5.0 | 6.9 | 7.3 | - | - | - | - |
| 10.0 | 7.1 | - | - | - | - | - |
| MMH | | | | | | |
| 1.0 | 7.0 | 7.2 | 7.3 | 7.4 | 7.4 | 7.4 |
| 2.5 | 6.8 | 7.1 | 7.2 | 7.3 | 7.4 | 7.4 |
| 5.0 | 6.9 | 7.1 | 7.3 | - | - | - |
| 7.5 | 6.9 | 7.1 | - | - | - | - |
| 10.0 | 6.9 | - | - | - | - | - |
| UDMH | | | | | | |
| 5 | 6.8 | 7.1 | 7.3 | 7.3 | 7.4 | 7.4 |
| 10 | 6.8 | 7.2 | 7.3 | 7.3 | 7.5 | 7.5 |
| 15 | 6.9 | 7.2 | 7.3 | 7.4 | 7.4 | 7.4 |
| 20 | 6.9 | 7.2 | 7.4 | 7.4 | 7.4 | 7.4 |
| 25 | 6.9 | 7.3 | 7.4 | 7.4 | 7.5 | 7.5 |
| Control | | | | | | |
| 1 | 6.8 | 7.0 | 7.2 | 7.2 | 7.1 | 7.1 |
| 2 | 6.7 | 6.9 | 7.2 | 7.3 | 7.2 | 7.2 |

TABLE 4 (Cont.)

Shiner (Trial 2)

| Compound | Time (hrs) | | | | | |
|-----------|------------------------------------|----------|-----------|-----------|-----------|-----------|
| | <u>Nominal Concentration (ppm)</u> | <u>0</u> | <u>24</u> | <u>48</u> | <u>72</u> | <u>96</u> |
| Hydrazine | | | | | | |
| .1 | | 6.7 | 6.9 | 7.1 | 7.4 | 7.6 |
| .5 | | 6.5 | 6.8 | 7.1 | 7.4 | 7.6 |
| 1.0 | | 6.6 | 7.0 | 7.1 | 7.4 | 7.7 |
| 3.0 | | 6.7 | 7.0 | 7.1 | 7.4 | - |
| 6.0 | | 6.8 | 7.1 | - | - | - |
| MMH | | | | | | |
| .1 | | 6.6 | 7.0 | 7.2 | 7.5 | 7.8 |
| .5 | | 6.7 | 6.9 | 7.2 | 7.4 | 7.7 |
| 1.0 | | 6.7 | 6.9 | 7.3 | 7.4 | 7.7 |
| 3.0 | | 6.7 | 7.0 | 7.3 | 7.5 | - |
| 5.0 | | 6.7 | 7.0 | 7.4 | - | - |
| UDMH | | | | | | |
| 20 | | 6.8 | 7.1 | 7.1 | - | 7.9 |
| 40 | | 6.9 | 7.1 | 7.2 | 7.5 | 7.7 |
| 60 | | 6.9 | 7.2 | 7.3 | 7.5 | 7.8 |
| 80 | | 7.1 | 7.2 | 7.3 | 7.6 | 7.8 |
| 100 | | 7.2 | 7.3 | 7.4 | - | 7.9 |
| Control | | | | | | |
| 1 | | 6.8 | 6.9 | 7.0 | 7.3 | 7.5 |
| 2 | | 6.7 | 6.9 | 7.0 | 7.3 | 7.5 |

TABLE 5

Preparation of Stock Solutions

| <u>Bioassay</u> | <u>Trial</u> | <u>Solute Volume</u> | | <u>Solute Plus Solvent</u> | <u>Final Conc.*</u> |
|-----------------|--------------|----------------------|----------|----------------------------|---------------------|
| Amphipod | 1 | H ₂ | 1.0 ml | 1.0 l | 1,000.0 |
| | | MMH | 1.0 ml | 1.0 l | 1,000.0 |
| | | UDMH | 1.0 ml | 100.0 l | 10,000.0 |
| | 2 | H ₂ | 1.0 ml | 1.0 l | 1,000.0 |
| | | MMH | 1.0 ml | 1.0 l | 1,000.0 |
| | | | | | |
| Isopod | 1 | H ₂ | 10.0 µl | 1.0 l | 10.0 |
| | | MMH | 10.0 µl | 1.0 l | 10.0 |
| | | UDMH | 1.0 ml | 100.0 ml | 10,000.0 |
| | 2 | MMH | 100.0 µl | 1.0 l | 100.0 |
| | | | | | |
| Catfish | 1 | H ₂ | 3.0 ml | 1.0 l | 3,000.0 |
| | | MMH | 3.0 ml | 1.0 l | 3,000.0 |
| | | UDMH | 6.0 ml | 1.0 l | 6,000.0 |
| | 2 | H ₂ | 3.0 ml | 1.0 l | 3,000.0 |
| | | MMH | 3.0 ml | 1.0 l | 3,000.0 |
| | | UDMH | 6.0 ml | 1.0 l | 6,000.0 |
| Shiner | 1 | H ₂ | 3.0 ml | 1.0 l | 3,000.0 |
| | | MMH | 3.0 ml | 1.0 l | 3,000.0 |
| | | UDMH | 6.0 ml | 1.0 l | 6,000.0 |
| | 2 | H ₂ | 3.0 ml | 1.0 l | 3,000.0 |
| | | MMH | 3.0 ml | 1.0 l | 3,000.0 |
| | | UDMH | 10.0 ml | 1.0 l | 10,000.0 |

* Concentrations expressed as ppm (V/V)..

TABLE 6

Toxicant Concentration During Bioassays

Amphipods (Trial 1)

| Compound | Nominal Concentration (ppm) | 0 hrs | 24 hrs | 48 hrs | % Loss ^a |
|----------|-----------------------------|--------|--------|--------|---------------------|
| Hz | | | | | |
| | 0.5 | 0.51 | 0.48 | b | |
| | 1.0 | 1.00 | 0.97 | b | |
| | 5.0 | 5.19 | b | | |
| | 10.0 | 17.22 | b | | |
| | 50.0 | 61.32 | b | | |
| MMH | | | | | |
| | 1.0 | 0.83 | 0.83 | b | 0 |
| | 5.0 | 4.84 | b | | |
| | 10.0 | 10.52 | b | | |
| | 50.0 | 61.87 | b | | |
| | 100.0 | 90.70 | b | | |
| UDMH | | | | | |
| | 5.0 | 5.00 | 1.28 | 1.18 | 76.4 |
| | 10.0 | 10.12 | 8.01 | 8.01 | 20.8 |
| | 25.0 | 41.94 | 32.91 | 25.88 | 38.3 |
| | 50.0 | 55.60 | c | b | |
| | 100.0 | 113.23 | 97.16 | b | 14.2 |

Amphipods (Trial 2)

| | | | | | |
|----|-----|-------------------|-------------------|-------------------|----|
| Hz | | | | | |
| | .01 | .005 ^d | .002 ^d | .002 ^d | 60 |
| | .05 | .04 | .03 | .02 | 50 |
| | .10 | .09 | .08 | .06 | 33 |
| | .25 | .25 | .23 | .20 | 20 |
| | .50 | .50 | .48 | .44 | 28 |

a Percent loss was determined from time intervals at which one or more organisms were alive.

b Concentrations were not determined, 100% mortality.

c Questionable data points.

d Extrapolation beyond calibration curve.

e Concentrations were not determined.

TABLE 6 (Cont.)

Amphipods (Trial 2, cont.)

| Compound Nominal Concentration (ppm) | 0 hrs | 24 hrs | 48 hrs | 72 hrs | % Loss |
|---|-------|--------|--------|--------|--------|
| MMH | | | | | |
| .01 | .02 | 0 | | | 100 |
| .05 | .05 | .04 | 0 | | 100 |
| .10 | .11 | .10 | 0 | | 100 |
| .25 | .23 | .22 | .07 | | 69.6 |
| .50 | .53 | .46 | .17 | | 67.9 |

Isopods (Trial 1)

Hz

| | | | | | |
|------|------|-------------------|------|-----|------|
| .01 | .01 | .001 ^d | | | 100 |
| .05 | .04 | .040 | .03 | .01 | 75 |
| .10 | .09 | .090 | .07 | .06 | 33.3 |
| .50 | .51 | .510 | .50 | .42 | 17.6 |
| 1.00 | 1.03 | 1.030 | 1.03 | .94 | 8.7 |

MMH

| | | | | | |
|------|------|------|------|--|------|
| .01 | .02 | .01 | 0 | | 50 |
| .05 | .05 | .02 | 0 | | 60 |
| .10 | .07 | .06 | .06 | | 14.3 |
| .50 | .50 | .49 | .46 | | 8 |
| 1.00 | 1.07 | 1.05 | 1.05 | | 2 |

UDMH

| | | | | | |
|-----|-------|-------|-------|--|------|
| 5 | 3.50 | 3.00 | 1.59 | | 54.6 |
| 10 | 8.30 | 7.58 | 6.68 | | 19.5 |
| 25 | 24.70 | 23.85 | 21.05 | | 14.8 |
| 50 | 51.60 | 48.80 | 45.40 | | 12.0 |
| 100 | 81.80 | 75.79 | 65.81 | | 19.6 |

Isopods (Trial 2)

MMH

| | | | | | |
|------|-------|-------|------|--|------|
| .05 | .10 | b | b | | 15.2 |
| .50 | .46 | b | .39 | | 24.2 |
| 1.00 | .91 | .91 | .69 | | 16.9 |
| 5.00 | 4.21 | 4.11 | 3.50 | | 13.3 |
| 10.0 | 9.25 | 9.04 | 8.02 | | 15.0 |
| 25.0 | 21.46 | 17.23 | b | | |

TABLE 6 (Cont.)

| Compound | Nominal Concentration (ppm) | 0 hrs | 24 hrs | 48 hrs | 72 hrs | 96 hrs | % Loss |
|-------------------|-----------------------------|-------|--------|--------|--------|--------|--------|
| Hz | | | | | | | |
| | .1 | .14 | 0 | 0 | 0 | 0 | 100 |
| | 1.0 | 1.67 | 1.35 | 1.32 | 1.32 | 1.24 | 25.8 |
| | 10.0 | 14.46 | b | | | | |
| | 25.0 | 24.80 | b | | | | |
| | 50.0 | 50.00 | b | | | | |
| MMH | | | | | | | |
| | .1 | .17 | .05 | .05 | .09 | .07 | 58.8 |
| | 1.0 | .84 | .78 | c | c | .69 | 17.8 |
| | 10.0 | 8.60 | 7.10 | b | | | |
| | 25.0 | 21.30 | b | | | | |
| | 50.0 | 49.20 | b | | | | |
| UDMH | | | | | | | |
| | 5 | 4.70 | 3.80 | c | 3.80 | 3.3 | 29.8 |
| | 10 | 8.00 | c | 7.50 | c | 7.5 | 6.2 |
| | 25 | 25.50 | 22.50 | 17.20 | b | | 32.5 |
| | 50 | 47.60 | 36.80 | b | | | 22.7 |
| | 100 | 93.50 | 90.20 | b | | | 3.5 |
| Catfish (Trial 2) | | | | | | | |
| Hz | | | | | | | |
| | .1 | e | | | | | |
| | .5 | .66 | .60 | e | e | 0.53 | 19.7 |
| | 1.0 | 1.46 | 1.43 | e | e | 1.20 | 17.8 |
| | 5.0 | 5.15 | b | | | | |
| | 10.0 | 15.38 | b | | | | |
| MMH | | | | | | | |
| | 1.0 | 1.23 | 1.19 | e | e | .89 | 27.6 |
| | 2.5 | 3.48 | 3.23 | e | e | c | |
| | 5.0 | 6.47 | 6.16 | e | b | | |
| | 7.5 | 11.25 | 9.42 | e | | | 16.3 |
| | 10.0 | 17.07 | 15.90 | e | | | 6.9 |
| UDMH | | | | | | | |
| | 5 | 5.61 | 4.95 | e | e | e | |
| | 10 | 15.02 | 8.80 | e | e | e | |
| | 15 | 17.92 | 14.54 | e | e | e | |
| | 20 | 23.18 | 19.61 | e | e | e | |
| | 25 | 28.26 | 24.41 | e | e | e | |

TABLE 6 (Cont.)

Shiner (Trial 1)

| Compound | | | | | | |
|-----------------------------|-------|--------|--------|--------|--------|--------|
| Nominal Concentration (ppm) | 0 hrs | 24 hrs | 48 hrs | 72 hrs | 96 hrs | % Loss |
| Hz | | | | | | |
| .1 | .09 | 0 | 0 | 0 | 0 | 100 |
| .5 | .31 | .31 | c | c | .31 | 0 |
| 1.0 | 1.59 | c | 1.59 | 1.51 | 1.00 | 37.1 |
| 5.0 | 5.80 | 4.98 | b | | | 14.1 |
| 10.0 | 15.89 | b | | | | |

MMH

| | | | | | | |
|------|------|------|---|------|------|-----|
| 1.0 | .81 | .54 | c | c | .49 | 9.2 |
| 2.5 | 2.17 | 2.17 | c | 2.07 | 2.07 | 4.6 |
| 5.0 | 4.64 | c | b | | | |
| 7.5 | 7.03 | b | | | | |
| 10.0 | 8.89 | b | | | | |

UDMH

| | | | | | | |
|----|-------|-------|-------|-------|-------|------|
| 5 | 5.27 | 3.97 | 3.97 | 3.86 | 2.45 | 53.5 |
| 10 | 10.82 | 9.96 | 9.96 | 9.31 | 9.20 | 15.0 |
| 15 | 16.16 | 15.73 | 15.73 | 14.45 | 11.92 | 26.2 |
| 20 | 22.48 | 21.83 | 21.83 | 20.20 | 19.76 | 12.1 |
| 25 | 22.05 | 18.56 | 18.46 | 14.31 | 14.31 | 34.1 |

Shiner (Trial 2)

Hz

| | | | | | | |
|-----|------|------|------|------|------|------|
| .1 | e | | | | | |
| .5 | .59 | .59 | .59 | .56 | .48 | 18.6 |
| 1.0 | 1.49 | 1.44 | 1.43 | 1.37 | 1.27 | 14.7 |
| 3.0 | 2.86 | 2.86 | 2.85 | 2.83 | b | 1.0 |
| 6.0 | 5.57 | 5.57 | b | | | 0.0 |

MMH

| | | | | | | |
|-----|------|------|------|------|------|-----|
| .1 | e | | | | | |
| .5 | .48 | .48 | .48 | .48 | .48 | 0 |
| 1.0 | 1.09 | 1.09 | 1.09 | 1.09 | 1.09 | 0 |
| 3.0 | 3.49 | 3.49 | 2.52 | c | b | |
| 6.0 | 5.91 | 5.91 | 5.74 | b | | 2.8 |

UDMH

| | | | | | | |
|-----|--------|-------|-------|------|------|------|
| 20 | 18.70 | 17.20 | 16.60 | b | | 11.2 |
| 40 | 39.80 | 37.00 | 36.50 | 34.6 | 34.6 | 13.0 |
| 60 | 58.60 | 57.00 | 55.40 | 55.4 | b | 5.4 |
| 80 | 77.60 | 75.00 | 72.40 | 72.2 | b | 6.9 |
| 100 | 100.20 | 94.00 | 88.60 | b | | 11.5 |

TABLE 7
Cumulative Percent Response

Amphipods (Trial 1)*

| Compound | | Time (hrs) | |
|----------|------------------------------------|------------|-----------|
| | <u>Nominal Concentration (ppm)</u> | <u>24</u> | <u>48</u> |
| Hz | | | |
| | .5 | 30 | 100 |
| | 1.0 | 90 | 100 |
| | 5.0 | 100 | |
| | 10.0 | 100 | |
| | 50.0 | 100 | |
| MMH | | | |
| | 1 | 10 | 100 |
| | 5 | 100 | |
| | 10 | 100 | |
| | 50 | 100 | |
| | 100 | 100 | |
| UDMH | | | |
| | 5 | 10 | 60 |
| | 10 | 15 | 60 |
| | 25 | 30 | 80 |
| | 50 | 30 | 100 |
| | 100 | 95 | 100 |
| Control | | | |
| | 1 | 20 | 45 |
| | 2 | 10 | 30 |

Amphipods (Trial 2)**

| | | | |
|-----|-----|----|----|
| Hz | | | |
| | .01 | 10 | 40 |
| | .05 | 10 | 40 |
| | .10 | 10 | 50 |
| | .25 | 20 | 60 |
| | .50 | 10 | 90 |
| MMH | | | |
| | .01 | 0 | 20 |
| | .05 | 20 | 30 |
| | .10 | 20 | 40 |
| | .25 | 10 | 20 |
| | .50 | 30 | 80 |

- * 20 individuals per concentration.
 ** 10 individuals per concentration.

TABLE 7 (Contd.)

Amphipods (Trial 2, cont)

| Compound | Time (hrs) | | |
|------------------------------------|------------|-----------|-----------|
| <u>Nominal Concentration (ppm)</u> | <u>24</u> | <u>48</u> | <u>72</u> |
| Control | | | |
| 1 | 0 | 20 | |
| 2 | 0 | 20 | |

Isopods (Trial 1)*

| | | | |
|-----|----|----|----|
| Hz | | | |
| .01 | 5 | 10 | 15 |
| .05 | 5 | 5 | 5 |
| .1 | 0 | 5 | 10 |
| .5 | 5 | 30 | 35 |
| 1.0 | 10 | 30 | 50 |

| | | | |
|-----|---|----|--|
| MMH | | | |
| .01 | 5 | 20 | |
| .05 | 5 | 15 | |
| .1 | 0 | 5 | |
| .5 | 0 | 0 | |
| 1.0 | 5 | 10 | |

| | | | |
|------|----|----|--|
| UDMH | | | |
| 5 | 10 | 10 | |
| 10 | 0 | 45 | |
| 25 | 5 | 80 | |
| 50 | 50 | 85 | |
| 100 | 75 | 90 | |

| | | | |
|---------|---|---|--|
| Control | | | |
| 1 | 0 | 0 | |
| 2 | 0 | 5 | |

Isopods (Trial 2)**

| | | | |
|---------|----|-----|--|
| MMH | | | |
| .05 | 10 | 30 | |
| .5 | 0 | 0 | |
| 1.0 | 30 | 60 | |
| 5.0 | 60 | 70 | |
| 10.0 | 70 | 90 | |
| 25.0 | 80 | 100 | |
| Control | 30 | 60 | |

* 20 individuals per concentration.

** 10 individuals per concentration.

TABLE 7 (Contd.)

Catfish (Trial 1)*

| Compound Nominal Concentration (ppm) | Time (hrs) | | | |
|---|------------|-----|-----|------|
| | 24 | 48 | 72 | 96 |
| Hz | | | | |
| .01 | 0 | 0 | 0 | 0 |
| 1.0 | 12.5 | 25 | 50 | 50 |
| 10.0 | 100 | | | |
| 25.0 | 100 | | | |
| 50 | 100 | | | |
| MMH | | | | |
| .1 | 0 | 0 | 0 | 0 |
| 1.0 | 0 | 0 | 0 | 0 |
| 10.0 | 62.5 | 100 | 100 | |
| 25.0 | 100 | | | |
| 50 | 100 | | | |
| UDMH | | | | |
| 5 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 87.5 |
| 25 | 0 | 50 | 100 | |
| 50 | 0 | 100 | | |
| 100 | 87.5 | 100 | | |
| Control | | | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |

Catfish (Trial 2)*

| | | | | |
|------|------|------|-----|------|
| Hz | | | | |
| .1 | 0 | 0 | 0 | 0 |
| .5 | 0 | 0 | 0 | 0 |
| 1.0 | 0 | 0 | 0 | 12.5 |
| 5.0 | 100 | | | |
| 10.0 | 100 | | | |
| MMH | | | | |
| 1.0 | 0 | 0 | 0 | 0 |
| 2.5 | 0 | 0 | 0 | 0 |
| 5.0 | 0 | 62.5 | 100 | |
| 7.5 | 12.5 | 100 | | |
| 10.0 | 75 | 100 | | |

* 8 individuals per concentration.

TABLE 7 (Cont.)

Catfish (Trial 2, cont.)

| Compound Nominal Concentration (ppm) | Time (hrs) | | | |
|---|------------|-----------|-----------|-----------|
| | <u>24</u> | <u>48</u> | <u>72</u> | <u>96</u> |
| UDMH | | | | |
| 5 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 12.5 |
| 15 | 0 | 0 | 25 | 50 |
| 20 | 0 | 12.5 | 50 | 50 |
| 25 | 0 | 0 | 50 | 100 |
| Control | | | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |

Shiner (Trial 1)*

| | | | | |
|---------|------|------|------|------|
| Hz | | | | |
| .1 | 0 | 0 | 0 | 0 |
| .5 | 0 | 0 | 0 | 0 |
| 1.0 | 0 | 0 | 12.5 | 37.5 |
| 5.0 | 87.5 | 100 | | |
| 10.0 | 100 | | | |
| MMH | | | | |
| 1.0 | 0 | 0 | 0 | 0 |
| 2.5 | 0 | 0 | 0 | 62.5 |
| 5.0 | 0 | 37.5 | 100 | |
| 7.5 | 37.5 | 100 | | |
| 10.0 | 87.5 | 100 | | |
| UDMH | | | | |
| 5 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 |
| Control | | | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |

Shiner (Trial 2)*

| | | | | |
|----|---|---|---|---|
| Hz | | | | |
| .1 | 0 | 0 | 0 | 0 |
| .5 | 0 | 0 | 0 | 0 |

* 8 individuals per concentration

TABLE 7 (Cont.)

Shiner (Trial 2, cont.)

| Compound Nominal Concentration (ppm) | Time (hrs) | | | |
|---|------------|-----------|-----------|-----------|
| | <u>24</u> | <u>48</u> | <u>72</u> | <u>96</u> |
| Hz | | | | |
| 1.0 | 0 | 0 | 0 | 0 |
| 3.0 | 0 | 25 | 87.5 | 100 |
| 6.0 | 62.5 | 100 | | |
| MMH | | | | |
| .1 | 0 | 0 | 0 | 0 |
| .5 | 0 | 0 | 0 | 0 |
| 1.0 | 0 | 0 | 0 | 0 |
| 3.0 | 0 | 0 | 87.5 | 100 |
| 5.0 | 0 | 75 | 100 | |
| UDMH | | | | |
| 20 | 0 | 0 | 100 | 87.5 |
| 40 | 0 | 0 | 0 | 100 |
| 60 | 0 | 0 | 37.5 | 100 |
| 80 | 0 | 0 | 75 | |
| 100 | 0 | 37.5 | 100 | |
| Control | | | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |

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